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(54) Laundry detergent composition.

(57) The compositions according to the present invention comprise conventional detergency ingredients, an alkaline cellulase and a polyvinylpyrrolidone. The combination of both these ingredients provide superior fabric colour care.

**EP 0 508 358 A1**

Technical field

The present invention relates to the laundering of fabrics. Laundry detergent compositions are disclosed which are specifically designed for the washing of coloured fabrics. The detergent compositions according to the present invention maintain the clarity of colours, especially upon repeated laundering.

Background

Laundry detergent compositions are well known in the art and it is known to use vinylpyrrolidone polymers in such compositions, as described in EP 262 897 and EP 256 696. It is also known to use such polymers in detergent compositions specifically designed for the washing of colored fabrics, as in EP-A-265 257 which discloses compositions containing a vinylpyrrolidone polymer, a carboxylate polymer and a carboxy methyl cellulose, and EP 372 291 which describes detergent compositions comprising a specific surfactant mixture and a vinyl pyrrolidone polymer. More specifically, it is known that vinyl pyrrolidone polymers act as dye transfer inhibitors in the laundering process, as described in DE 28 14 287 and DE 28 14 329.

It is also known to use enzymes in laundry detergent compositions, including cellulase. Cellulase is known to have some effect on maintaining colours of fabrics inasmuch as cellulase controls the pilling of fabrics. This is described for instance in EP 177 165.

It has now been found that superior fabric colour maintenance could be achieved upon laundering by using a detergent composition comprising both a polyvinylpyrrolidone and cellulase; the combined effect of both these ingredients has surprisingly been found to be superior than the addition of the effects obtained by either alone.

Summary of the invention

The compositions according to the invention are laundry detergent compositions comprising conventional detergency ingredients, characterized in that they comprise an alkaline cellulase at a level in the finished product so as to deliver from 0.005 to 40 mg/l of the wash solution of said cellulase, and a polyvinylpyrrolidone of a molecular weight of from 5000 to 1000000 at a level in the finished product so as to deliver from 5 to 500 mg/l of said polyvinylpyrrolidone in the wash solution.

Detailed description of the invention.

The laundering detergent compositions according to the present invention comprise conventional detergency ingredients, including surfactants, builders and minor ingredients.

Suitable surfactants for use in the compositions according to the present invention include anionic surfactants such as water-soluble salts of alkyl benzene sulphonates, alkyl sulphates, alkyl polyethoxy ether sulphates, paraffin sulphonates, alpha-olefin sulphonates, alpha-sulphoalkylcarboxylates and their esters, alkyl glyceryl ether sulphonates, fatty acid monoglyceride sulphates and sulphonates, alkyl phenol polyethoxy ether sulphates, 2-acyloxy-alkane-1-sulphonates, and betaalkyloxy sulphonates.

Especially preferred alkyl benzene sulphonates have 9 to 15 carbon atoms in a linear or branched alkyl chain, especially from 11 to 13 carbon atoms. Suitable alkyl sulphates have from 10 to 22 carbon atoms in the alkyl chain, more especially from 12 to 18 carbon atoms. Suitable alkyl polyethoxy ether sulphates have from 10 to 18 carbon atoms in the alkyl chain and have an average of from 1 to 23 - CH<sub>2</sub>CH<sub>2</sub>O-groups per molecule, especially from 10 to 16 carbon atoms in the alkyl chain and an average of from 1 to 6 - CH<sub>2</sub>-O groups per molecule.

Suitable paraffin sulphonates are essentially linear and contain from 8 to 24 atoms, more especially from 14 to 18 carbon atoms. Suitable alpha-olefin sulphonates have from 10 to 24 carbon atoms, more especially from 14 to 16 carbon atoms; alpha-olefin sulphonates can be made by reaction with sulphur trioxide, followed by neutralization under conditions such that any sulfones present are hydrolyzed to the corresponding hydroxy alkane sulphonates. Suitable alpha-sulphocarboxylates contain from 6 to 20 carbon atoms; included herein are not only the salts of alpha-sulphonated fatty acids but also their esters made from alcohols containing 1 to 14 carbon atoms.

Suitable alkyl glyceryl ether sulphates are ethers of alcohols having from 10 to 18 carbon atoms, more especially those derived from coconut oil and tallow. Suitable alkyl phenol polyethoxy ether sulphates have from 8 to 12 carbon atoms in the alkyl chain and an average of from 1 to 6 - CH<sub>2</sub>CH<sub>2</sub>O-groups per molecule. Suitable 2-acyloxyalkane-1-sulphonates contain from 2 to 9 carbon atoms in the acyl group and

from 9 to 23 carbon atoms in the alkane moiety. Suitable beta-alkyloxy alkane sulphonates contain from 1 to 3 carbon atoms in the alkyl group and from 8 to 20 carbon atoms in the alkane moiety.

Nonionic surfactants suitable for use in the compositions herein, are water-soluble ethoxylated materials of HLB 11.5-17.0 and include C<sub>10-20</sub> primary and secondary alcohol ethoxylates and C<sub>6-10</sub> alkylphenol ethoxylates. C<sub>14-18</sub> linear primary alcohols condensed with from seven to thirty moles of ethylene oxide per mole of alcohol are preferred examples being C<sub>14-C15</sub>(EO)<sub>7</sub>, C<sub>16-18</sub>(EO)<sub>25</sub> and especially C<sub>16-18</sub>(EO)<sub>11</sub>.

Another class of nonionic surfactants comprises alkyl polyglucoside compounds of general formula

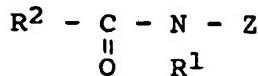


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wherein Z is a moiety derived from glucose; R is an saturated hydrophobic alkyl group that contains from 12 to 18 carbon atoms; t is from 0 to 10 and n is 2 or 3; x is from 1.3 to 4, the compounds including less than 10% unreacted fatty alcohol and less than 50% short chain alkyl polyglucosides. Compounds of this type and their use in detergent are disclosed in EP-B 0 070 077, 0 075 996 and 0 094 118.

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Also suitable as nonionic surfactants are poly hydroxy fatty acid amide surfactants of the formula



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wherein R<sup>1</sup> is H, C<sub>1-4</sub> hydrocarbyl, 2-hydroxy ethyl, 2-hydroxy propyl or a mixture thereof, R<sup>2</sup> is C<sub>5-31</sub> hydrocarbyl, and Z is a polyhydroxyhydrocarbyl having a linear hydrocarbyl chain with at least 3 hydroxyls directly connected to the chain, or an alkoxylated derivative thereof. Preferably, R<sup>1</sup> is methyl, R<sup>2</sup> is a straight C<sub>11-15</sub> alkyl or alkenyl chain such as coconut alkyl or mixtures thereof, and Z is derived from a reducing sugar such as glucose, fructose, maltose, lactose, in a reductive amination reaction.

Other types of surfactants can be used, such as zwitterionic amphoteric, as well as cationic surfactants.

Cationic co-surfactants which can be used herein, include water-soluble quaternary ammonium compounds of the form R<sub>4</sub>R<sub>5</sub>R<sub>6</sub>R<sub>7</sub>N<sup>+</sup>X<sup>-</sup>, wherein R<sub>4</sub> is alkyl having from 10 to 20, preferably from 12-18 carbon atoms, and R<sub>5</sub>, R<sub>6</sub> and R<sub>7</sub> are each C<sub>1</sub> to C<sub>7</sub> alkyl preferably methyl; X<sup>-</sup> is an anion, e.g. chloride. Examples of such trimethyl ammonium compounds include C<sub>12-14</sub> alkyl trimethyl ammonium chloride and cocalkyl trimethyl ammonium methosulfate. The compositions according to the present invention comprise from 1-70% by weight of surfactant, preferably from 10% to 30%, most preferably from 15%-25%.

35

### Builders

Suitable builders for use herein include the nitrilotriacetates, polycarboxylates, citrates, water-soluble phosphates such as tri-polyphosphate and sodium ortho- and pyro-phosphates, and mixtures thereof. Metal ion sequestrants include all of the above, plus materials like ethylenediaminetetraacetate, the aminopolyphosphonates and a wide variety of other poly-functional organic acids and salts too numerous to mention in detail here. See U.S. Patent 3,579,454 for typical examples of the use of such materials in various cleaning compositions. Preferred polyfunctional organic acids species for use herein are citric acid, ethylene diamine tetramethylenephosphonic acid, and diethylene triaminepentamethylenephosphonic acid.

A further class of detergency builder materials useful in the present invention are insoluble sodium aluminosilicates. The 1-10 micron size zeolite (e.g., zeolite A) builder disclosed in German Patent 24 22 655 are especially preferred for use in low-phosphate compositions.

The compositions herein can also contain fatty acids, saturated or unsaturated, and the corresponding soaps. Suitable fatty acids, saturated or unsaturated, have from 10 to 18 carbon atoms in the alkyl chain. Preferred are unsaturated species having from 14 to 18 carbon atoms in the alkyl chain, most preferably oleic acid. The corresponding soaps can also be used.

The compositions herein can also contain compounds of the general formula R-CH(COOH)CH<sub>2</sub>(COOH) i.e. derivatives of succinic acid, wherein R is C<sub>10-C20</sub> alkyl or alkenyl, preferably C<sub>12-C16</sub> or wherein R may be substituted with hydroxyl, sulfo, sulfoxyl or sulfone substituents.

The succinate builders are preferably used in the form of their water-soluble salts, including the sodium, potassium, ammonium and alkanolammonium salts.

Specific examples of succinate builders include : lauryl succinate, myristyl succinate, palmityl succinate, 2-dodecetyl succinate (preferred), 2-pentadecenyl succinate, and the like.

Also useful as builders in the present context are the compounds described in US Patent 4,663,071, i.e. mixtures of tartrate monosuccinic acid and tartrate disuccinic acid in weight ratio of monosuccinic to disuccinic of from 97:3 to 20:80, preferably 95:5 to 40:6.

Compositions according to the present invention comprise from 1% to 70% of a builder, preferably from 5 30% to 60%, most preferably from 40% to 50%.

The compositions according to the present invention are characterized in that they comprise an alkaline cellulase and a polyvinylpyrrolidone. It is this specific combination of ingredients which provides the superior fabric colour care properties of the compositions according to the present invention. These fabric colour care benefits are better obtained when the fabrics are repeatedly washed with the compositions 10 according to the present invention - thus the present invention also compasses a method of washing fabrics wherein the fabrics are repeatedly washed with a composition according to the present invention.

#### The cellulase

15 The cellulase usable in the present invention may be any bacterial or fungal cellulase, having a pH optimum of between 5 and 9.5.

Suitable cellulase are disclosed in GB-A-2 075 028;

GB-A-2 095 275 and DE-OS-24 47 832.

Examples of such cellulases are cellulase produced by a strain of *Humicola insolens* (*Humicola grisea* 20 var. *thermoidea*), particularly by the *Humicola* strain DSM 1800, and cellulases produced by a fungus of *Bacillus N* or a cellulase 212-producing fungus belonging to the genus *Aeromonas*, and cellulase extracted from the hepatopancreas of a marine mullosc (*Dolabella Auricula Solander*).

The cellulase added to the composition of the invention may be in the form of a non-dusting granulate, e.g. "marumes" or "prills", or in the form of a liquid in which the cellulase is provided as a cellulase 25 concentrate suspended in e.g. a nonionic surfactant or dissolved in an aqueous medium.

Preferred cellulases for use herein are characterized in that said cellulase they provide at least 10% removal of immobilized radioactive labelled carboxymethylcellulose according to the C14CMC-method at  $25 \times 10^{-6}$ % by weight of cellulase protein in the laundry test solution.

Other suitable water-soluble organic salts are the homo- or co-polymeric acids or their salts, in which 30 the polycarboxylic acid comprises at least two carboxyl radicals separated from each other by not more than two carbon atoms.

Polymers of this type are disclosed in GB-A-1,596,756. Examples of such salts are polyacrylates of MW 2000-5000 and their copolymers with maleic anhydride, such copolymers having a molecular weight of from 20,000 to 70,000, especially about 40,000.

#### CELLULASE

The activity of enzymes and particularly the activity of cellulase enzyme has been defined for various 40 applications by different analytical methods. These methods all attempt to provide a realistic assessment of the expected in use performance or at least a measurement correlating with the in use performance. As has been detailed in European Patent Application EP-A-350098, many of the methods, particularly these frequently used by cellulase manufacturers, are not sufficiently correlated with the in use performance of cellulase in laundry detergent compositions. This is due to the various other usage conditions for which these activity measurement methods have been developed.

45 The method described in EP-A-350098, has been developed to be and to have a predictive correlation for the ranking of cellulase activity in laundry detergent compositions.

The present invention therefore uses the method disclosed in EP-A-350098 to screen cellulases in order to distinguish cellulases which are useful in the present invention and those which would not provide the objectives of the present invention. The screening method, hereinafter referred to as C14CMC-Method, 50 which has been adopted from the method disclosed in EP-A-350098, can be described as follows:

#### Principle :

The principle of the C14CMC-Method for screening is to measure at a defined cellulase concentration in 55 a wash solution the removal of immobilized carboxy methyl cellulose (CMC) from a cloth substrate. The removal of CMC is measured by radio-active labelling of some of the CMC by using C14 radio-active carbon. Simple counting of the amount of radio-active C14 on the cloth substrate before and after the cellulase treatment allows the evaluation of the cellulase activity.

Sample preparation :

**CMC preparation :** The radio-active CMC stock solution is prepared according to Table I. The radio-active CMC can be obtained by methods referred to in EP-A-350098.

5 **Fabric substrates :** The fabric substrates are muslin cotton swatches having a size of 5 cm x 5 cm. They are inoculated with 0.35 ml of the radio-active labelled CMC stock solution in their center. The muslin cotton swatches are then air-dried.

10 **Immobilization of CMC :** To immobilize the radio-active labelled CMC on the muslin cotton swatches, laundrometer equipment " Linitest Original Haunau " made by Original Haunau, Germany, is used. A metal jar of the laundrometer is filled with 400 ml of hard water (4 mmol/liter of Ca<sup>++</sup> ions). A maximum number of 13 swatches can be used per jar. The jar is then incubated in a heat-up cycle from 20 °C to 60 °C over 40 minutes in the laundrometer equipment. After incubation the swatches are rinsed under running city water for 1 minute. They are squeezed and allowed to air-dry for at least 30 minutes.

15 According to EP-A-350098 samples of the swatches with immobilized radio-active CMC can also be measured as "blank samples" without washing.

Sample treatment :

20 **Laundry test solution :** The laundry test solution is prepared according to the composition of Table II. It is balanced to pH 7.5. The laundry test solution is the basis to which a cellulase test sample is added. Care should be taken to not dilute the laundry test solution by adding water to a 100% balance prior to having determined the amount of cellulase to be added. The amount of cellulase which is used in this screening test should be added to provide  $25 \times 10^{-6}$  weight percent of cellulase protein in the laundry test solution (equivalent to 0.25 milligram/liter at 14.5 °C).

25 **Wash procedure :** The swatches thus inoculated with radio-active labelled CMC are then treated in a laundry simulation process. The laundry process is simulated in the laundrometer type equipment, " Linitest, Original Haunau ", by Original Haunau, Haunau, Germany. An individual swatch is put into a 20 cm<sup>3</sup> glass vial. The vial is filled with 10 ml of the laundry test solution and then sealed liquid tight. Up to 5 vials are put into each laundrometer jar. The jar is filled with water as a heat transfer medium for the laundering simulation. The laundering simulation is conducted as a heat-up cycle from 20 °C to 60 °C over 40 minutes.

30 After the processing of the samples the vials are submerged in cold water and subsequently each swatch is taken out of its vial, rinsed in a beaker under running soft water, squeezed and allowed to air-dry for at least 30 minutes.

35 Measurement :

40 In order to measure radio-active labelled CMC removal, a scintillation counter, for example, a LKB 1210 Ultrabeta Scintillation Counter, is used. In order to obtain most accurate results, the instruction manual for optimum operation of the particular scintillation counter should be followed. For example, for the LKB 1210 Ultrabeta Scintillation Counter, the following procedure should be followed. The swatch to be measured is put into a plastic vial filled with 12 ml of scintillator liquid (e.g. scintillator 299 from Packard). The swatch is then allowed to stabilize for at least 30 minutes. The vial is then put into the LKB 1210 Ultrabeta Scintillation Counter and the respective radio-activity counts for the swatch is obtained.

45 In order to measure the amount of CMC removal due only to the cellulase, a measurement of a swatch which has been inoculated at the same time but has been treated in the laundry test solution without cellulase, is necessary. The activity of the cellulase is then expressed as percent of radio-active labelled CMC removal. This percentage is calculated by the following formula :

$$50 \quad \% \text{ of radio-active CMC removal} = \frac{X_0 - X_C}{X_0} \times 100$$

55 Wherein

X<sub>0</sub> is the radioactivity scintillation count of a swatch treated with the laundry test solution without cellulase

X<sub>C</sub> is the radioactivity scintillation count of a swatch treated with the laundry test solution containing the

cellulase to be evaluated

**Statistical considerations, procedure confirmation :**

5 In order to provide statistically sound results, standard statistical analysis should be employed. For the given example, using the LKB 1210 Ultrabeta Scintillation Counter, it has been found that a sample size of 3 swatches for each radioactivity scintillation count can be used.

In order to confirm the procedure by internal crosschecking, measurement and calculation of the "blank sample" according to EP-A-350098 are recommended. This will allow to detect and eliminate errors.

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**Interpretation of results :**

The described screening test does provide a fast, unique and reliable method to identify cellulases which satisfy the activity criteria of the present invention versus cellulases which are not part of the present invention.

15 It has been found that a removal of 10% or more of the immobilized radioactive labelled CMC according to the above C14CMC-method, indicates that the respective cellulase satisfies the requirements of the invention.

20 It will be obvious to those skilled in the art that removal percentages above 10% indicate a higher activity for the respective cellulase. It therefore is contemplated that cellulase providing above 25% or preferably above 50% removal of radioactive labelled CMC, at the protein concentration in the laundry test solution according to the C14CMC-method, would provide indication of an even better performance of the cellulase for use in laundry detergents.

25 It also has been contemplated that usage of higher concentrations of cellulase for C14CMC-method, would provide higher removal percentages. However, there exists no linear proven correlation between cellulase concentration and removal percentage obtained by it.

It also has been contemplated that usage of higher concentrations of cellulase for C14CMC-method, would provide higher removal percentages.

30

TABLE I

Radioactive C <sub>14</sub> labelled CMC stock solution (all percentages by weight of total solution)	
35 Total CMC* (CMC should be detergent grade CMC with a degree of substitution from about 0.47 to about 0.7)	99.2 x 10 <sup>-3</sup> %
Ethanol	14985.12 x 10 <sup>-3</sup> %
Deionized Water	84915.68 x 10 <sup>-3</sup> %
40 Total :	100%

\* Total CMC contains non-radio-active and radio-active CMC to provide a radio-activity which allows sufficiently clear readings on the scintillation counter used.  
For example, the radio-active CMC can have an activity of 0.7 millicurie/g and be mixed

50

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TABLE II

Laundry test solution (all percentages by weight of total solution)		
5	Linear C <sub>12</sub> alkyl benzene sulphonic acid	0.110%
10	Coconut alkyl sulphate (TEA salt)	0.040%
15	C <sub>12-15</sub> alcohol ethoxylate (E07)	0.100%
20	Coconut fatty acid	0.100%
25	Oleic acid	0.050%
30	Citric acid	0.010%
35	Triethanolamine	0.040%
40	Ethanol	0.060%
45	Propanediol	0.015%
50	Sodium hydroxide	0.030%
55	Sodium formate	0.010%
60	Protease	0.006%
65	Water (2.5 mmol/liter Ca <sup>++</sup> ), pH adjustment agent (HCl or NaOH solutions) and cellulase	balance to 100%

According to the present invention, preferred cellulases are those as described in International Patent Application WO 91/17243. For example, a cellulase preparation useful in the compositions of the invention can consist essentially of a homogeneous endoglucanase component, which is immunoreactive with an antibody raised against a highly purified 43kD cellulase derived from Humicola insolens, DSM 1800, or which is homologous to said 43kD endoglucanase.

It should be stressed that all cellulase enzymes according to the present invention have to meet the criteria of the above mentioned screening test. However, in the Danish Patent Application 1159/90 additional criteria are established allowing to identify preferred cellulase enzymes in combination with the present screening test.

Cellulase preparations particularly useful in the compositions of the invention are those in which in addition to the screening test, the endoglucanase component exhibits a CMC-endoase activity of at least about 50, preferably at least about 60, in particular at least about 90 CMC-endoase units per mg of total protein. In particular, a preferred endoglucanase component exhibits a CMC-endoase activity of at least 100 CMC-endoase units per mg of total protein.

In the present context, the term "CMC-endoase activity" refers to the endoglucanase activity of the endoglucanase component in terms of its ability to degrade cellulose to glucose, cellobiose and triose, as determined by a viscosity decrease of a solution of carboxymethyl cellulose (CMC) after incubation with the cellulase preparation of the invention, as described in detail below.

The CMC-endoase (endoglucanase) activity can be determined from the viscosity decrease of CMC, as follows : A substrate solution is prepared, containing 35 g/l CMC (Hercules 7 LFD) in 0.1 M tris buffer at pH 9.0. The enzyme sample to be analyzed is dissolved in the same buffer. 10 ml substrate solution and 0.5 ml enzyme solution are mixed and transferred to a viscosimeter (e.g. Haake VT 181, NV sensor, 181 rpm), thermostated at 40 °C. Viscosity readings are taken as soon as possible after mixing and again 30 minutes later. The amount of enzyme that reduces the viscosity to one half under these conditions is defined as 1 unit of CMC-endoase activity.

SDS polyacrylamide gel electrophoresis (SDS-PAGE) and isoelectric focusing with marker proteins in a manner known to persons skilled in the art were used to determine the molecular weight and isoelectric point (pI), respectively, of the endoglucanase component in the cellulase preparation useful in the present context. In this way, the molecular weight of a specific endoglucanase component was determined to be 43kD. The isoelectric point of this endoglucanase was determined to be about 5.1.

The cellobiohydrolase activity may be defined as the activity towards cellobiose p-nitrophenyl. The activity is determined as umole nitrophenyl released per minute at 37 °C and pH 7.0. The present endoglucanase component was found to have essentially no cellobiohydrolase activity.

The endoglucanase component in the cellulase preparation herein has initially been isolated by extensive purification procedures, i.a. involving reverse phase HPLC purification of a crude H. insolens cellulase mixture according to U.S. 4,435,307. This procedure has surprisingly resulted in the isolation of a 43kD endoglucanase as a single component with unexpectedly favourable properties due to a surprisingly high endoglucanase activity.

Also, in addition to the screening test, the cellulase enzymes useful in the present compositions can further be defined as enzymes exhibiting endoglucanase activity (in the following referred to as an "endoglucanase enzyme"), which enzymes have the amino acid sequence shown in the appended Sequence Listing ID#2, or a homologue thereof exhibiting endoglucanase activity.

In the present context, the term "homologue" is intended to indicate a polypeptide encoded by DNA which hybridizes to the same probe as the DNA coding for the endoglucanase enzyme with this amino acid sequence under certain specified conditions (such as presoaking in 5xSSC and prehybridizing for 1 h at 40 °C in a solution of 20% formamide, 5xDenhardt's solution, 50 mM sodium phosphate, pH 6.8, and 50 ug of denatured sonicated calf thymus DNA, followed by hybridization in the same solution supplemented with 100 uM ATP for 18 h at 40 °C). The term is intended to include derivatives of the aforementioned sequence obtained by addition of one or more amino acid residues to either or both the C- and N-terminal of the native sequence, substitution of one or more amino acid residues at one or more sites in the native sequence, deletion of one or more amino acid residues at either or both ends of the native amino acid sequence or at one or more sites within the native sequence, or insertion of one or more amino acid residues at one or more sites in the native sequence.

The endoglucanase enzyme herein may be one producible by species of Humicola such as Humicola insolens e.g. strain DSM 1800, deposited on October 1, 1981 at the Deutsche Sammlung von Mikroorganismen, Mascheroder Weg 1B, D-3300 Braunschweig, FRG, in accordance with the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure (the Budapest Treaty).

In still a further aspect, the cellulase enzymes useful herein can be defined, in addition to the screening test, as endoglucanase enzymes which have the amino acid sequence shown in the appended Sequence Listing ID#4, or a homologue thereof (as defined above) exhibiting endoglucanase activity. Said endoglucanase enzyme may be one producible by a species of Fusarium, such as Fusarium oxysporum, e.g. strain DSM 2672, deposited on June 6, 1983 at the Deutsche Sammlung von Mikroorganismen, Mascheroder Weg 1B, D-3300 Braunschweig, FRG, in accordance with the provisions of the Budapest Treaty.

Furthermore, it is contemplated that homologous endoglucanases may be derived from other microorganisms producing cellulolytic enzymes, e.g. species of Trichoderma, Myceliphthora, Phanerochaete, Schizophyllum, Penicillium, Aspergillus, and Geotrichum.

For industrial production of the cellulase preparation herein, however, it is preferred to employ recombinant DNA techniques or other techniques involving adjustments of fermentations or mutation of the microorganisms involved to ensure overproduction of the desired enzymatic activities. Such methods and techniques are known in the art and may readily be carried out by persons skilled in the art.

The endoglucanase component may thus be one which is producible by a method comprising cultivating a host cell transformed with a recombinant DNA vector which carries a DNA sequence encoding said endoglucanase component or a precursor of said endoglucanase component as well as DNA sequences encoding functions permitting the expression of the DNA sequence encoding the endoglucanase component or precursor thereof, in a culture medium under conditions permitting the expression of the endoglucanase component or precursor thereof and recovering the endoglucanase component from the culture.

DNA constructs comprising a DNA sequence encoding an endoglucanase enzyme as described above, or a precursor form of the enzyme, include the DNA constructs having a DNA sequence as shown in the appended Sequence Listings ID#1 or ID#3, or a modification thereof. Examples of suitable modifications of the DNA sequence are nucleotide substitutions which do not give rise to another amino acid sequence of the endoglucanase, but which correspond to the codon usage of the host organism into which the DNA construct is introduced or nucleotide substitutions which do give rise to a different amino acid sequence and therefore, possibly, a different protein structure which might give rise to an endoglucanase mutant with different properties than the native enzyme. Other examples of possible modifications are insertion of one or more nucleotides at either end of the sequence, or deletion of one or more nucleotides at either end or within the sequence.

DNA constructs encoding endoglucanase enzymes useful herein may be prepared synthetically by established standard methods, e.g. the phosphoamidite method described by S.L. Beaucage and M.H.

Caruthers, Tetrahedron Letters 22, 1981, pp. 1859-1869, or the method described by Matthes et al., EMBO Journal 3, 1984, pp. 801-805. According to the phosphoamidite method, oligonucleotides are synthesized, e.g. in an automatic DNA synthesizer, purified, annealed, ligated and cloned in suitable vectors.

A DNA construct encoding the endoglucanase enzyme or a precursor thereof may, for instance, be isolated by establishing a cDNA or genomic library of a cellulase-producing microorganism, such as Humicola insolens, DSM 1800, and screening for positive clones by conventional procedures such as by hybridization using oligonucleotide probes synthesized on the basis of the full or partial amino acid sequence of the endoglucanase in accordance with standard techniques (cf. Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd. Ed. Cold Spring Harbor, 1989), or by selecting for clones expressing the appropriate enzyme activity (i.e. CMC-endoase activity as defined above), or by selecting for clones producing a protein which is reactive with an antibody against a native cellulase (endoglucanase).

Finally, the DNA construct may be of mixed synthetic and genomic, mixed synthetic and cDNA or mixed genomic and cDNA origin prepared by ligating fragments of synthetic, genomic or cDNA origin (as appropriate), the fragments corresponding to various parts of the entire DNA construct, in accordance with standard techniques. The DNA construct may also be prepared by polymerase chain reaction using specific primers, for instance as described in US 4,683,202 or R.K. Saiki et al., Science 239, 1988, pp. 487-491.

Recombinant expression vectors into which the above DNA constructs are inserted include any vector which may conveniently be subjected to recombinant DNA procedures, and the choice of vector will often depend on the host cell into which it is to be introduced. Thus, the vector may be an autonomously replicating vector, i.e. a vector which exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, e.g. a plasmid. Alternatively, the vector may be one which, when introduced into a host cell, is integrated into the host cell genome and replicated together with the chromosome(s) into which it has been integrated.

In the vector, the DNA sequence encoding the endoglucanase should be operably connected to a suitable promoter and terminator sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. The procedures used to ligate the DNA sequences coding for the endoglucanase, the promoter and the terminator, respectively, and to insert them into suitable vectors are well known to persons skilled in the art (cf., for instance, Sambrook et al., op.cit.).

Host cells which are transformed with the above DNA constructs or the above expression vectors may be for instance belong to a species of Aspergillus, most preferably Aspergillus oryzae or Aspergillus niger. Fungal cells may be transformed by a process involving protoplast formation and transformation of the protoplasts followed by regeneration of the cell wall in a manner known per se. The use of Aspergillus as a host microorganism is described in EP 238 023 (of Novo Industri A/S), the contents of which are hereby incorporated by reference. The host cell may also be a yeast cell, e.g. a strain of Saccharomyces cerevisiae.

Alternatively, the host organism may be a bacterium, in particular strains of Streptomyces and Bacillus, and E. coli. The transformation of bacterial cells may be performed according to conventional methods, e.g. as described in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, 1989.

The screening of appropriate DNA sequences and construction of vectors may also be carried out by standard procedures, cf. Sambrook et al., op.cit.

The medium used to cultivate the transformed host cells may be any conventional medium suitable for growing the host cells in question. The expressed endoglucanase may conveniently be secreted into the culture medium and may be recovered therefrom by well-known procedures including separating the cells from the medium by centrifugation or filtration, precipitating proteinaceous components of the medium by means of a salt such as ammonium sulphate, followed by chromatographic procedures such as ion exchange chromatography, affinity chromatography, or the like.

By employing recombinant DNA techniques as indicated above, techniques of protein purification, techniques of fermentation and mutation or other techniques which are well known in the art, it is possible to provide endoglucanases of a high purity.

The level in the present composition of cellulase described above should be such that the amount of enzyme protein to be delivered in the wash solution is from 0.005 to 40 mg/liter of wash solution, preferably 0.01 to 10 mg/liter of wash solution.

##### 55 The vinylpyrrolidone polymer

The composition according to the invention also comprises a polyvinylpyrrolidone having a molecular weight of from 5000 to 1 000 000, preferably from 5000 to 50000, most preferably from 8000 to 15000. The

level in the compositions according to the present invention of polyvinylpyrrolidone should be such that the amount of polyvinylpyrrolidone delivered in the wash solution is from 5 to 500 mg/l, preferably from 15 to 100 mg/l, most preferably 25 mg/l to 75 mg/l.

5    Optional ingredients

The present composition will typically include optional ingredients that normally form part of detergent compositions. Antiredeposition and soil suspension agents, optical brighteners, clays, bleaches, bleach activators, suds suppressors, anticaking agents, dyes, perfumes and pigments are examples of such optional ingredients and can be added in varying amounts as desired.

10    The compositions according to the invention can be in liquid, pasty or granular form, preferably granular. Granular compositions according to the present invention can also be in "compact form", i.e. they may have a relatively higher density than conventional granular detergents, i.e. from 550 to 950 g/l; in such case, the granular detergent compositions according to the present invention will contain a lower amount of "inorganic filler salt", compared to conventional granular detergents; typical filler salts are alkaline ether metal salts of sulphates and chlorides, typically sodium sulphate; "compact" detergents typically comprise not more than 10% filler salt. Liquid and conventional granular detergents are typically used at a concentration of from 1 to 2% by weight in the wash liquor, preferably 1.5%, whereas compact granules are used at a concentration of from 0.5% to 1.5% by weight, preferably 1%.

15    20    The following examples illustrate the present invention and the unexpected superior colour care benefits obtained therefrom.

EXAMPLE I

25    The following composition was made:

30	Linear alkyl benzene sulphonate (LAS)	11%
	Alkyl sulphate	5%
	Nonionic	6%
	Trisodium citrate	15%
	Zeolite	32%
	Polymer	4%
	Chelant	0.2%
35	Sodium sulphate	5%
	Sodium silicate	2%

A load consisting of two types of coloured fabric (blue knit 91% cotton/9% polyester, and a peach coloured 100% cotton flannel material) was washed 10 times with this composition at a concentration in the wash liquor of 0.7%. In the 10th cycle an additional fabric was introduced which bled dye into the wash. In one experiment this was a red cotton fabric, in another this was a brown cotton fabric. Colour appearance on the washed load was then evaluated using a panel score with the following scale :

- 1. I think I see a difference with the reference
  - 2. I definitely see a difference with the reference
  - 45    3. I see a big difference with the reference
  - 4. A day and night difference with the reference
- The above detergent composition was then supplemented either with 1% PVP, or with 0.025% cellulase, or with both 0.025% cellulase and 1% PVP. Colour appearance was again evaluated as above. Results are summarized in the table below.
- 50    These results show that the action of PVP and cellulase together is unexpectedly superior than the sum of the individual actions of both these ingredients.

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OVERALL COLOUR APPEARANCE BENEFITS - PVP + CELLULASE - PANEL SCORE UNITS

		Blue Knit (91% Cotton, 9% Polyester)		Peach Flannel (100% Cotton)	
		Red Dye Transfer	Brown Dye Transfer	Red Dye Transfer	Brown Dye Transfer
Reference - Aged Fabric at Start	No PVP, No Cellulase	-2.2	-4.0	-2.5	-4.0
	With Cellulase, No PVP	1.8	-3.5	2.5	-4.0
	With PVP, No Cellulase	-1.2	-2.0	-0.2	-0.8
	With PVP, With Cellulase	1.5	1.0	3.5	1.5
Reference - New Fabric at Start	No PVP, No Cellulase	-3.2	-4.0	-2.2	-4.0
	With Cellulase, No PVP	-1.5	-4.0	-1.8	-4.0
	With PVP, No Cellulase	-2.8	-3.0	-1.5	-3.0
	With PVP, With Cellulase	-1.2	-1.5	-0.5	0.0
Reference - Aged Fabric Washed w/o PVP and w/o Cellulase	With Cellulase, No PVP	2.8	1.8	1.8	0.2
	With PVP, No Cellulase	0.5	3.5	1.0	3.2
	With PVP, With Cellulase	4.0	3.8	4.0	4.0

55 EXAMPLES II to VII

The following compositions are made.

- a) Compact granular detergent : examples II and III

	EXAMPLES	II	III
5	Linear alkyl benzene sulphonate	11.40	10.70
	Tallow alkyl sulphate	1.80	2.40
	C <sub>12</sub> alkyl sulphate	3.00	3.10
	C <sub>12</sub> alcohol 7 times ethoxylated	4.00	4.00
	Tallow alcohol 11 times ethoxylated	1.80	1.80
	Dispersant	0.07	0.1
	Silicone fluid	0.80	0.80
10	Trisodium citrate	14.00	15.00
	Citric acid	3.00	2.50
	Zeolite	32.50	32.10
	Maleic acid acrylic acid copolymer	5.00	5.00
	DETMPA	1.00	0.20
15	Cellulase (active protein)	0.03	0.025
	Alkalase/BAN	0.60	0.60
	Lipase	0.36	0.40
	Sodium silicate	2.00	2.50
	Sodium sulphate	3.50	5.20
20	PVP	0.30	0.50

b) conventional granular detergent : examples IV and V

	EXAMPLES	IV	V
25	Sodium linear C <sub>12</sub> alkyl benzene sulfonate	6.5	8.0
	Sodium sulfate	15.0	18.0
	Zeolite A	26.0	22.0
30	Sodium nitrilotriacetate	5.0	5.0
	Cellulase (active protein)	0.02	0.03
	PVP	0.5	0.7
	TAED	3.0	3.0
	Boric acid	4.0	-
35	Minors	up to 100	

c) liquid detergent : examples VI and VII

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EXAMPLES	VI	VII
C <sub>12-14</sub> alkenyl succinic acid	3.0	8.0
Citric acid monohydrate	10.0	15.0
Sodium C <sub>12-15</sub> alkyl sulphate	8.0	8.0
Sodium sulfate of C <sub>12-15</sub> alcohol 2 times ethoxylated	-	3.0
C <sub>12-15</sub> alcohol 7 times ethoxylated	-	8.0
C <sub>12-15</sub> alcohol 5 times ethoxylated	8.0	-
Diethylene triamine penta (methylene phosphonic acid)	0.2	-
Oleic acid	1.8	-
Ethanol	4.0	4.0
Propanediol	2.0	2.0
Protease	0.2	0.2
Cellulase (active protein)	0.2	0.05
PVP	1.0	2.0
Suds suppressor	0.15	0.15
NaOH	up to pH 7.5	
Waters and minors	up to 100 parts	

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**INFORMATION FOR SEQ ID NO 1 :**

### (i) SEQUENCE CHARACTERISTICS

- (A) Length : 1060 base pairs
  - (B) Type : nucleic acid
  - (C) Strandedness : single
  - (D) Topology : linear

(ii) MOLECULE TYPE : cDNA

(iii) HYPOTHETICAL : NO

**(iv) ORIGINAL SOURCE**

- (A) Organism : *Humicola insolens*  
(B) Strain : DSM 1800

(ix) FEATURE

- (A) Name/Key : mat\_peptide  
(B) Location : 73..927

(ix) FEATURE

- (A) Name/Key : sig\_peptide  
(B) Location : 10..72

(ix) FEATURE

- (A) Name/Key : CDS  
(B) Location : 10..927

30

SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGATCCAAG ATG CGT TCC TCC CCC CTC CTC CCG TCC GCC GTT GTG GCC	48
Met Arg Ser Ser Pro Leu Leu Pro Ser Ala Val Val Ala	
-21 -20 -15 -10	
GCC CTG CCG GTG TTG GCC CTT GCC GCT GAT GGC AGG TCC ACC CGC TAC	96
Ala Leu Pro Val Leu Ala Leu Ala Asp Gly Arg Ser Thr Arg Tyr	
-5 1 5	
TGG GAC TGC TGC AAG CCT TCG TGC GGC TGG GCC AAG AAG GCT CCC GTG	144
Trp Asp Cys Cys Lys Pro Ser Cys Gly Trp Ala Lys Lys Ala Pro Val	
10 15 20	
AAC CAG CCT GTC TTT TCC TGC AAC GCC AAC TTC CAG CGT ATC ACG GAC	192
Asn Gln Pro Val Phe Ser Cys Asn Ala Asn Phe Gln Arg Ile Thr Asp	
25 30 35 40	
TTC GAC CCC AAG TCC GGC TGC GAG CCG GGC GGT GTC GCC TAC TCG TGC	240
Phe Asp Ala Lys Ser Gly Cys Glu Pro Gly Gly Val Ala Tyr Ser Cys	
45 50 55	
GCC GAC CAG ACC CCA TGG GCT GTG AAC GAC GAC TTC GCG CTC GGT TTT	288
Ala Asp Gln Thr Pro Trp Ala Val Asn Asp Asp Phe Ala Leu Gly Phe	
60 65 70	

	GCT GCC ACC TCT ATT GCC GGC AGC AAT GAG GCG GGC TGG TGC TGC GCC Ala Ala Thr Ser Ile Ala Gly Ser Asn Glu Ala Gly Trp Cys Cys Ala 75 80 85	336
5	TGC TAC GAG CTC ACC TTC ACA TCC GGT CCT GTT GCT GGC AAG AAG ATG Cys Tyr Glu Leu Thr Phe Thr Ser Gly Pro Val Ala Gly Lys Lys Met 90 95 100	384
10	GTC GTC CAG TCC ACC AGC ACT GGC GGT GAT CTT GGC AGC AAC CAC TTC Val Val Gln Ser Thr Ser Thr Gly Gly Asp Leu Gly Ser Asn His Phe 105 110 115 120	432
	GAT CTC AAC ATC CCC GGC GGC GTC GGC ATC TTC GAC GGA TGC ACT Asp Leu Asn Ile Pro Gly Gly Val Gly Ile Phe Asp Gly Cys Thr 125 130 135	480
15	GCC CAG TTC GGC GGT CTG CCC GGC CAG CGC TAC GGC GGC ATC TCG TCC Pro Gln Phe Gly Gly Leu Pro Gly Gln Arg Tyr Gly Gly Ile Ser Ser 140 145 150	528
20	CGC AAC GAG TGC GAT CGG TTC CCC GAC GCC CTC AAG CCC GGC TGC TAC Arg Asn Glu Cys Asp Arg Phe Pro Asp Ala Leu Lys Pro Gly Cys Tyr 155 160 165	576
	TGG CGC TTC GAC TGG TTC AAG AAC GAC AAT CCG AGC TTC AGC TTC Trp Arg Phe Asp Trp Phe Lys Asn Ala Asp Asn Pro Ser Phe Ser Phe 170 175 180	624
25	CGT CAG GTC CAG TGC CCA GCC GAG CTC GTC GCT CGC ACC GGA TGC CGC Arg Gln Val Gln Cys Pro Ala Glu Leu Val Ala Arg Thr Gly Cys Arg 185 190 195 200	672
	CGC AAC GAC GAC GGC AAC TTC CCT GCC GTC CAG ATC CCC TCC AGC AGC Arg Asn Asp Asp Gly Asn Phe Pro Ala Val Gln Ile Pro Ser Ser Ser 205 210 215	720
30	ACC AGC TCT CCG GTC AAC CAG CCT ACC AGC ACC AGC ACC ACG TCC ACC Thr Ser Ser Pro Val Asn Gln Pro Thr Ser Thr Ser Thr Thr Ser Thr 220 225 230	768
35	TCC ACC ACC TCG AGC CCG CCA GTC CAG CCT ACG ACT CCC AGC GGC TGC Ser Thr Thr Ser Ser Pro Pro Val Gln Pro Thr Thr Pro Ser Ser Gly Cys 235 240 245	816
	ACT GCT GAG AGG TGG GCT CAG TGC GGC GGC AAT GGC TGG AGC GGC TGC Thr Ala Glu Arg Trp Ala Gln Cys Gly Gly Asn Gly Trp Ser Gly Cys 250 255 260	864
40	ACC ACC TGC GTC CCT GGC AGC ACT TGC ACG AAG ATT AAT GAC TGG TAC Thr Thr Cys Val Ala Gly Ser Thr Cys Thr Lys Ile Asn Asp Trp Tyr 265 270 275 280	912
45	CAT CAG TGC CTG TAGACGCCAGG GCAGCTTGAG GGCCCTTAATG GTGGCCCGCAA His Gln Cys Leu 285	964
	CGAAATGACA CTCCCAATCA CTGTATTAGT TCTTGTACAT AATTCGTCA TCCCTCCAGG	1024
	GATTGTCACA TAAATGCAAT GAGGAACAAT GAGTAC	1060
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## INFORMATION FOR SEQ ID NO 2 :

## (i) SEQUENCE CHARACTERISTICS

- 5  
 (A) Length : 305 amino acids  
 (B) Type : amino acid  
 (D) Topology : linear

## (ii) MOLECULE TYPE : protein

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## SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Arg Ser Ser Pro Leu Leu Pro Ser Ala Val Val Ala Ala Leu Pro  
 15 -21 -20 -15 -10

Val Leu Ala Leu Ala Ala Asp Gly Arg Ser Thr Arg Tyr Trp Asp Cys  
 5 -5 1 5 10

Cys Lys Pro Ser Cys Gly Trp Ala Lys Lys Ala Pro Val Asn Gln Pro  
 20 15 20 25

Val Phe Ser Cys Asn Ala Asn Phe Gln Arg Ile Thr Asp Phe Asp Ala  
 30 35 40

Lys Ser Gly Cys Glu Pro Gly Val Ala Tyr Ser Cys Ala Asp Gln  
 25 45 50 55

Thr Pro Trp Ala Val Asn Asp Asp Phe Ala Leu Gly Phe Ala Ala Thr  
 30 60 65 70 75

Ser Ile Ala Gly Ser Asn Glu Ala Gly Trp Cys Cys Ala Cys Tyr Glu  
 35 80 85 90

Leu Thr Phe Thr Ser Gly Pro Val Ala Gly Lys Lys Met Val Val Gln  
 40 95 100 105

Ser Thr Ser Thr Gly Gly Asp Leu Gly Ser Asn His Phe Asp Leu Asn  
 45 110 115 120

Ile Pro Gly Gly Val Gly Ile Phe Asp Gly Cys Thr Pro Gln Phe  
 50 125 130 135

Gly Gly Leu Pro Gly Gln Arg Tyr Gly Gly Ile Ser Ser Arg Asn Glu  
 55 140 145 150 155

Cys Asp Arg Phe Pro Asp Ala Leu Lys Pro Gly Cys Tyr Trp Arg Phe  
 60 160 165 170

Asp Trp Phe Lys Asn Ala Asp Asn Pro Ser Phe Ser Phe Arg Gln Val  
 65 175 180 - 185

Gln Cys Pro Ala Glu Leu Val Ala Arg Thr Gly Cys Arg Arg Asn Asp  
 70 190 195 200

Asp Gly Asn Phe Pro Ala Val Gln Ile Pro Ser Ser Ser Thr Ser Ser  
 75 205 210 215

5	Pro Val Asn Gln Pro Thr Ser Thr Ser Thr Thr Ser Thr Ser Thr Thr 220                    225                    230                    235
10	Ser Ser Pro Pro Val Gln Pro Thr Thr Pro Ser Gly Cys Thr Ala Glu 240                    245                    250
15	Arg Trp Ala Gln Cys Gly Gly Asn Gly Trp Ser Gly Cys Thr Thr Cys 255                    260                    265
20	Val Ala Gly Ser Thr Cys Thr Lys Ile Asn Asp Trp Tyr His Gln Cys 270                    275                    280
	Leu

INFORMATION FOR SEQ ID NO 3

### (i) SEQUENCE CHARACTERISTICS

- (A) LENGTH : 1473 base pairs
  - (B) TYPE : nucleic acid
  - (C) STRANDEDNESS : single
  - (D) TOPOLOGY : linear

- (ii) MOLECULE TYPE : cDNA

- (iii) HYPOTHETICAL : NO

- (iv) ANTI-SENSE : NO

- (vi) ORIGINAL SOURCE  
(A) ORGANISM : *fusarium oxysporum*  
(B) STRAIN : DSM 2672

- ### (ix) FEATURE

- (A) NAME/KEY : CDS  
(B) LOCATION : 97..1224

SEQUENCE DESCRIPTION: SEQ ID NO:3:

	AAC CCC ATT TCC AAC ACC AAT GCT GTC AAC GGT TGT GAG GGT GGT Asn Pro Ile Ser Asn Thr Asn Ala Val Asn Gly Cys Glu Gly Gly 55 60 65 70	306
	TCT GCT TAT GCT TGC ACC AAC TAC TCT CCC TGG GCT GTC AAC GAT GAG Ser Ala Tyr Ala Cys Thr Asn Tyr Ser Pro Trp Ala Val Asn Asp Glu 75 80 85	354
10	CTT GCC TAC GGT TTC GCT GCT ACC AAG ATC TCC GGT GGC TCC GAG GCC Leu Ala Tyr Gly Phe Ala Ala Thr Lys Ile Ser Gly Gly Ser Glu Ala 90 95 100	402
	AGC TGG TGC TGT GCT TAT GCT TTG ACC TTC ACC ACT GGC CCC GTC Ser Trp Cys Cys Ala Cys Tyr Ala Leu Thr Phe Thr Thr Gly Pro Val 105 110 115	450
20	AAG GGC AAG AAG ATG ATC GTC CAG TCC ACC AAC ACT GGA GGT GAT CTC Lys Gly Lys Lys Met Ile Val Gln Ser Thr Asn Thr Gly Gly Asp Leu 120 125 130	498
	GGC GAC AAC CAC TTC GAT CTC ATG ATG CCC GGC GGT GGT GTC GGT ATC Gly Asp Asn His Phe Asp Leu Met Met Pro Gly Gly Val Gly Ile 135 140 145 150	546
25	TTC GAC GGC TGC ACC TCT GAG TTC GGC AAG GCT CTC GGC GGT GCC CAG Phe Asp Gly Cys Thr Ser Glu Phe Gly Lys Ala Leu Gly Gly Ala Gln 155 160 165	594
30	TAC GGC GGT ATC TCC TCC CGA AGC GAA TGT GAT AGC TAC CCC GAG CTT Tyr Gly Gly Ile Ser Ser Arg Ser Glu Cys Asp Ser Tyr Pro Glu Leu 170 175 180	642
	CTC AAG GAC GGT TGC CAC TGG CGA TTC GAC TGG TTC GAG AAC GCC GAC Leu Lys Asp Gly Cys His Trp Arg Phe Asp Trp Phe Glu Asn Ala Asp 185 190 195	690
35	AAC CCT GAC TTC ACC TTT GAG CAG GTT CAG TGC CCC AAG GCT CTC CTC Asn Pro Asp Phe Thr Phe Glu Gln Val Gln Cys Pro Lys Ala Leu Leu 200 205 210	738
40	GAC ATC AGT GGA TGC AAG CCG GAT GAC GAC TCC AGC TTC CCT GCC TTC Asp Ile Ser Gly Cys Lys Arg Asp Asp Ser Ser Phe Pro Ala Phe 215 220 225 230	786
	AAG GTT GAT ACC TCG GCC AGC AAG CCC CAG CCC TCC AGC TCC GCT AAG Lys Val Asp Thr Ser Ala Ser Lys Pro Gln Pro Ser Ser Ser Ala Lys 235 240 245	834
45	AAG ACC ACC TCC GCT GCT GCT GCC GCT CAG CCC CAG AAG ACC AAG GAT Lys Thr Thr Ser Ala Ala Ala Ala Gln Pro Gln Lys Thr Lys Asp 250 255 260	882
50	TCC GCT CCT GTT GTC CAG AAG TCC ACC AAG CCT GCC GCT CAG CCC Ser Ala Pro Val Val Gln Lys Ser Ser Thr Lys Pro Ala Ala Gln Pro 265 270 275	930

	GAG CCT ACT AAG CCC GCC GAC AAG CCC CAG ACC GAC AAG CCT GTC GCC Glu Pro Thr Lys Pro Ala Asp Lys Pro Gin Thr Asp Lys Pro Val Ala 280 285 290	978
5	ACC AAG CCT GCT ACC AAG CCC GTC CAA CCT GTC AAC AAG CCC AAG Thr Lys Pro Ala Ala Thr Lys Pro Val Gin Pro Val Asn Lys Pro Lys 295 300 305 310	1026
10	ACA ACC CAG AAG GTC CGT GGA ACC AAA ACC CGA GGA AGC TGC CCG GCC Thr Thr Gin Lys Val Arg Gly Thr Lys Thr Arg Gly Ser Cys Pro Ala 315 320 325	1074
15	AAG ACT GAC GCT ACC GCC AAG GCC TCC GTT GTC CCT GCT TAT TAC CAG Lys Thr Asp Ala Thr Ala Lys Ala Ser Val Val Pro Ala Tyr Tyr Gin 330 335 340	1122
20	TGT GGT GGT TCC AAG TCC GCT TAT CCC AAC GGC AAC CTC GCT TGC GCT Cys Gly Gly Ser Lys Ser Ala Tyr Pro Asn Gly Asn Leu Ala Cys Ala 345 350 355	1170
25	ACT GGA AGC AAG TGT GTC AAG CAG AAC GAG TAC TAC TCC CAG TGT GTC Thr Gly Ser Lys Cys Val Lys Gin Asn Glu Tyr Tyr Ser Gin Cys Val 360 365 370	1218
30	CCC AAC TAAATGGTAG ATCCATCGGT TGTGGAAGAG ACTATGGTC TCAGAAGGGA Pro Asn 375	1274
	TCCTCTCATG AGCAGGCTTC TCATTGTATA GCATGGCATC CTGGACCAAG TGTTGACACC TTGTTGTACA TAGTATATCT TCATTGTATA TATTTAGACA CATAGATAGC CTCTTGTCAAG CGACAACTGG CTACAAAAGA CTTGGCAGGC TTGTTCAATA TTGACACAGT TTCTTCCATA AAAAAAAAA AAAAAAAA	1334 1394 1454 1473
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**INFORMATION FOR SEQ ID NO 4**

### (i) SEQUENCE CHARACTERISTICS

- (A) LENGTH : 376 amino acids
  - (B) TYPE : amino acid
  - (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein

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SEQUENCE DESCRIPTION: SEQ ID NO:4:

15	Met Arg Ser Tyr Thr Leu Leu Ala Leu Ala Gly Pro Leu Ala Val Ser
	1 5 10 15
20	Ala Ala Ser Gly Ser Gly His Ser Thr Arg Tyr Trp Asp Cys Cys Lys
	20 25 30
25	Pro Ser Cys Ser Trp Ser Gly Lys Ala Ala Val Asn Ala Pro Ala Leu
	35 40 45
30	Thr Cys Asp Lys Asn Asp Asn Pro Ile Ser Asn Thr Asn Ala Val Asn
	50 55 60
35	Gly Cys Glu Gly Gly Ser Ala Tyr Ala Cys Thr Asn Tyr Ser Pro
	65 70 75 80
40	Trp Ala Val Asn Asp Glu Leu Ala Tyr Gly Phe Ala Ala Thr Lys Ile
	85 90 95
45	Ser Gly Gly Ser Glu Ala Ser Trp Cys Cys Ala Cys Tyr Ala Leu Thr
	100 105 110
50	Phe Thr Thr Gly Pro Val Lys Gly Lys Lys Met Ile Val Gln Ser Thr
	115 120 125
55	Asn Thr Gly Gly Asp Leu Gly Asp Asn His Phe Asp Leu Met Met Pro
	130 135 140
60	Gly Gly Gly Val Gly Ile Phe Asp Gly Cys Thr Ser Glu Phe Gly Lys
	145 150 155 160
65	Ala Leu Gly Gly Ala Gln Tyr Gly Gly Ile Ser Ser Arg Ser Glu Cys
	165 170 175
70	Asp Ser Tyr Pro Glu Leu Leu Lys Asp Gly Cys His Trp Arg Phe Asp
	180 185 190
75	Trp Phe Glu Asn Ala Asp Asn Pro Asp Phe Thr Phe Glu Gln Val Gln
	195 200 205

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Cys Pro Lys Ala Leu Leu Asp Ile Ser Gly Cys Lys Arg Asp Asp Asp  
 210 215 220  
 5 Ser Ser Phe Pro Ala Phe Lys Val Asp Thr Ser Ala Ser Lys Pro Gln  
 225 230 235 240  
 10 Pro Ser Ser Ser Ala Lys Lys Thr Thr Ser Ala Ala Ala Ala Gln  
 245 250 255  
 Pro Gln Lys Thr Lys Asp Ser Ala Pro Val Val Gln Lys Ser Ser Thr  
 260 265 270  
 15 Lys Pro Ala Ala Gln Pro Glu Pro Thr Lys Pro Ala Asp Lys Pro Gln  
 275 280 285  
 Thr Asp Lys Pro Val Ala Thr Lys Pro Ala Ala Thr Lys Pro Val Gln  
 290 295 300  
 20 Pro Val Asn Lys Pro Lys Thr Thr Gln Lys Val Arg Gly Thr Lys Thr  
 305 310 315 320  
 Arg Gly Ser Cys Pro Ala Lys Thr Asp Ala Thr Ala Lys Ala Ser Val  
 325 330 335  
 25 Val Pro Ala Tyr Tyr Gln Cys Gly Gly Ser Lys Ser Ala Tyr Pro Asn  
 340 345 350  
 Gly Asn Leu Ala Cys Ala Thr Gly Ser Lys Cys Val Lys Gln Asn Glu  
 355 360 365  
 30 Tyr Tyr Ser Gln Cys Val Pro Asn  
 370 375

**Claims**

- 35 1. A laundry detergent composition comprising conventional detergency ingredients, characterized in that it comprises an alkaline cellulase at a level in the finished product so as to deliver from 0.005 to 40 mg/l of the wash solution of said cellulase, and a polyvinylpyrrolidone of a molecular weight of from 5000 to 100000 at a level in the finished product so as to deliver from 5 to 500 mg/l of said polyvinylpyrrolidone in the wash solution.
- 40 2. A laundry detergent composition according to claim 1 wherein said cellulase provides at least 10% removal of immobilized radioactive labelled carboxymethylcellulose according to the C14CMC-method at  $25 \times 10^{-6}$ % by weight of cellulase protein in the laundry test solution,
- 45 said detergent composition containing no more than 15% by weight of inorganic filler salt, and said detergent composition having a density of 550 to 950 g/litre of composition.
- 50 3. A laundry detergent composition according to claim 2 characterized in that the cellulase consists essentially of a homogeneous endoglucanase component which is immunoreactive with a monoclonal antibody raised against a partially purified about  $\approx$  43kD cellulase derived from Humicola insolens, DSM 1800, or which is homologous to said  $\approx$  43kD endoglucanase.
- 55 4. A laundry detergent composition according to the preceding claims wherein the cellulase is present at a level in the finished product so as to deliver from 0.01 mg/l to 10 mg/l of cellulase in the wash liquor.
- 55 5. A laundry detergent composition according to any of the preceding claims wherein the polyvinylpyrrolidone has a molecular weight of from 5000 to 50000, preferably 8000 to 15000.

6. A detergent composition according to any of the preceding claims wherein the polyvinylpyrrolidone is present at a level so as to deliver from 15 mg/1 to 100 mg/1 of polyvinylpyrrolidone in the wash solution, preferably from 25 mg/1 to 75 mg/1.
- 5 7. A laundry detergent composition according to any of the preceding claims, wherein said conventional detergency ingredients consists of surfactants and builders.
8. A laundry detergent composition according to any of the preceding claims which is in a granular form.
- 10 9. A laundry detergent composition according to claim 7 which has a density of from 550 g/1 to 950 g/1.
10. A method of washing fabrics, wherein said fabrics are repeatedly washed with a detergent compositions according to any of the preceding claims.

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European Patent  
Office

## EUROPEAN SEARCH REPORT

Application Number

EP 92 10 5956

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
D, X	US-A-4 435 307 (P. BARBESGAARD) * column 7, line 7 - line 18 * * example 7 * -----	1,5-8,10	C11D3/386 C11D3/37
A	WO-A-8 909 259 (NOVO INDUSTRI) * page 11, line 7 - line 37; claims 1,8,10,18,24,25 * -----	1-3	
A	CHEMICAL ABSTRACTS, vol. 111, no. 26, 26 April 1989, Columbus, Ohio, US; abstract no. 235677, 'JP-A-1132698 (LION)' page 150 ; * abstract *	1-3,8,9	
A	GB-A-2 094 826 (KAO SOAP) * page 13, line 46 - line 49; claim 1; example 3 * -----	1	
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D,A	EP-A-0 372 291 (HENKEL) *page 4, line 19 - line 24* * page 3, line 41 - line 51; claims * -----	1,5-7	TECHNICAL FIELDS SEARCHED (Int. Cl.5)
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<p>The present search report has been drawn up for all claims</p>			
Place of search THE HAGUE	Date of compilation of the search 13 JULY 1992	Examiner PFANNENSTEIN H.	
CATEGORY OF CITED DOCUMENTS		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons A : member of the same patent family, corresponding document	
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document			